Multiplex analysis of pro-inflammatory cytokines and pig-Major Acute Phase Protein in plasma of lipopolysaccharide-challenged pigs

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Introduction

Lipopolysaccharide (LPS) has been widely used as a model of immune challenge in pigs.[1,2,3,4,5] It provokes the synthesis of pro-inflammatory cytokines, such as tumor necrosis factor-alpha (TNF-α), interleukin-1β (IL-1β) and IL-6, which trigger the acute phase response by inducing fever and stimulating hepatocytes to produce acute phase proteins.[6] Pig-Major Acute Phase Protein (pig-MAP) is an excellent biomarker of different pathologies in pigs.[7] Although time-consuming and expensive, Enzyme-Linked Immuno Sorbent Assays (ELISAs) are typically used for the determination of individual cytokines and acute phase proteins in plasma.[3,5] However, there is growing interest in the simultaneous detection of multiple analytes in small samples by multiplex particle based flow cytometry.[8] Two such immunoassays have been reported in pigs: one for TNF-α, IL-1β and IL-8 and one for TNF-α, IL-6, IL-8 and IL-10.[8,9] We aimed to develop a new multiplex method for porcine TNF-α, IL-1β, IL-6 and pig-MAP.

Materials and Methods

Capture antibodies (abs) were covalently linked to the surface of different color-coded 7.5 µm polystyrene Functional Beads (Becton Dickinson, BD). A Lightning-Link R-Phycoerythrin (R-PE) conjugation kit (Innova Biosciences) was used for the R-PE conjugation of detection abs. A mixture of beads was incubated for 30 min with an appropriate standard mixture. Subsequently, a mixture of detection abs was added and incubated for 2 h. Finally, strepavidin-PE (R&D Systems) was added and samples were analyzed on a BD FACSArray® flow cytometer. Four stress resistant pigs (Seghers Hybrid®) were challenged intravenously with 15 µg ultrapure LPS/kg body weight (*E. coli* serotype O111:B4, Cayla-InvivoGen), two control pigs received the equivalent volume of 0.9% NaCl. Rectal body temperature was measured and blood samples were collected several times until 72 h p.a. Plasma samples were also analyzed with bio-assays (Vlaams Instituut voor Biotechnologie, VIB) and ELISAs (R&D Systems and PigCHAMP Pro Europe S.A.).

Results and Discussion

For the first time, TNF-α, IL-1β, IL-6 and pig-MAP were measured simultaneously. Four representative standard curves were created after the multiplex procedure and successfully compared to the singleplex graphs. Appropriate optimization steps, regarding sample dilutions, wash- and incubation procedures, and suitable antibody pairs were performed. The ELISA and bio-assay results will be used to compare with the multiplex data. A pronounced rise in body temperature (BT) was observed after LPS administration: the $AUC_{0\rightarrow 24h}$ of the mean BT time curve of 2 control and 4 LPS-challenged pigs was 931.3 ± 0.27 and 944.5 ± 0.57 (°Cxh), respectively. The ultimate purpose of this research is to study immunomodulatory effects of antibiotics, steroidal and non-steroidal drugs in this *in vivo* inflammatory pig model. After validation, the new multiplex method will be a powerful tool for the quantification of pro-inflammatory cytokines and pig-MAP in LPS-challenged pigs treated with these drugs. Results of the newly developed multiplex method on plasma samples from the pigs will be presented.

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